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EDGEWOOD ARSENAL
TECHNICAL REPORT

EATR 4565

DESIGN AND SYNTHESIS OF HEMICHOLINIUMS AND QUATERNARY AMMONIUM COMPOUNDS WITH RELATIVELY GREAT DIFFERENCES BETWEEN THE EFFECTIVE AND LETHAL DOSES

PA 40, by 496

Harold Z. Sommer
Omer O. Owens

November 1971

DEPARTMENT OF THE ARMY
EDGEWOOD ARSENAL
Research Laboratories
Chemical Research Laboratory
Edgewood Arsenal, Maryland 21010

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Organic Chemistry Department

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Task 1B562607AD12

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FOREWORD

The work described in this report was performed under Task 1B562607AD12, Chemical Agents, Incapacitating Agents. The experimental data are recorded in notebooks 7123, 7125, 7194, 7536, 7920, 8085, and 8094. The work was started in December 1963 and completed in February 1971.

Acknowledgments

The authors are indebted to Mr. R. D. Deibel for his valuable assistance in the experimental work, to Mr. C. A. Rush, Mr. J. M. Corliss, Mr. S. S. Cruikshank, Mr. E. J. W. Rhodes, Mrs. M. F. Buckles, Mrs. N. B. Scholtz, and Mrs. S. Hammel, Microanalytical Branch, Analytical Chemistry Department, for the microanalyses; to Dr. R. G. Horton, Mr. W. J. Lennox, Dr. A. H. McCreesh, Mr. T. A. Koviak, Mr. M. B. Shutz, and Miss P. Moore, Toxicity Screening Branch, Organic Chemistry Department, for the toxicological data.

DIGEST

Nine selected series of quaternary ammonium compounds were synthesized to study relationships between their chemical structures and biological activities. A general structure is presented indicating certain common chemical features required to obtain potent hemicholiniums, lethal pyridinium compounds, and other pyridinium compounds with relatively great differences between the effective and lethal doses. A new hemicholinium compound (EA 5236), more potent than the original hemicholinium (HC-3), was obtained. General chemical characteristics regarding the compositions of the presynaptic active sites participating in cholinergic neuromuscular impulse transmission are postulated. Possible mechanisms of action of *ortho*- and *meta*-pyridinium compounds meeting specific structural requirements are proposed.

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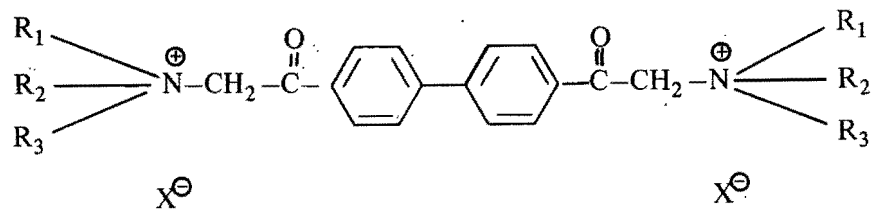
DESIGN AND SYNTHESIS OF HEMICHOLINIUMS AND QUATERNARY AMMONIUM COMPOUNDS WITH RELATIVELY GREAT DIFFERENCES BETWEEN THE EFFECTIVE AND LETHAL DOSES

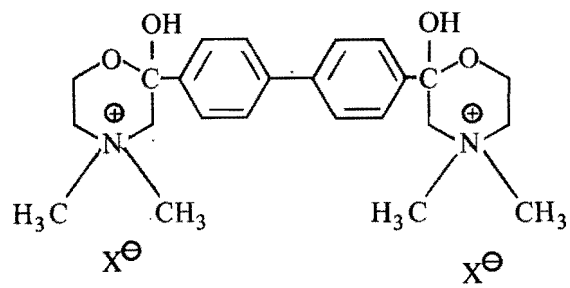
I. INTRODUCTION.

Long and Schueler[1] reported in 1954 the syntheses, toxicities, and acetylcholinesterase inhibition data of a number of bis-quaternary ammonium compounds of the following general formula A. The compounds have in common a biphenacyl chain connecting the onium centers. The different analogs vary in the substitutions on the positively charged nitrogens.



A

The toxicities of the 11 compounds prepared did not parallel acetylcholinesterase (AChE) inhibiting potencies; surprisingly, the dimethylethanolammonium derivative [$R_1R_2 = -CH_3$, $R_3 = -(CH_2)_2OH$], which was the least active acetylcholinesterase inhibitor, was found to be the most lethal compound with an ip LD50 of about 120 μg/kg in mice. On the basis of infrared spectra that showed no absorption in the carbonyl region but contained strong hydroxyl bands, Schueler[2] demonstrated that in the course of synthesis, the compound undergoes cyclization to the dihemiketal of structure B.



B

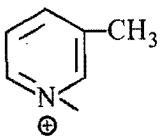
In light of the bis-choline-like character of structure B, Schueler named the compound hemicholinium (HC-3). Pharmacologically, the most striking characteristic of HC-3 was its ability to cause respiratory paralysis only after a latent period lasting many minutes, or even hours, depending on the animal species tested. It was clear that in exerting this effect, HC-3 was not acting in the manner of the parasympathetic blocking agents. Respiratory failure was not accompanied by neuromuscular blockade or any other familiar parasympatholytic symptoms unless the doses were above the LD50 levels.

The lethal potency of HC-3 coupled with its unfamiliar pharmacological effects provided the impetus for investigators interested in cholinergic mechanisms of impulse transmission to

[1] Long, J. P., and Schueler, F. W. J. Amer. Pharm. Ass. *43*, 79 (1954).
[2] Schueler, F. W. J. Pharmacol. Exp. Ther. *115*, 127 (1955).

explore the mode of action of HC-3 more thoroughly. Furthermore, closely related analogs of HC-3, designated as the hemicholiniums, were synthesized[3-8] and became available for pharmacological evaluation. Schueler[2] proposed that the primary toxic action of the hemicholiniums was directed against some pathway involved in the control of the respiratory muscles, and that the locus of respiratory failure was in the cervical relay centers for respiration in the spinal cord. In later years it has been shown that, in addition to their central activities, the hemicholiniums interfere also with cholinergic transmission in the peripheral nervous system. A considerable amount of evidence accumulated indicating that the hemicholiniums act presynaptically at the ganglia,[9-13] as well as at the neuromuscular junctions,[14-16] and that they inhibit acetylcholine biosynthesis at some stage before acetylation of choline by choline acetylase (acetyl-CoA: choline O-acetyltransferase). Furthermore, it is postulated that HC-3 like materials reduce the release of acetylcholine from the efferent nerve terminals, and that they also might affect the concentrating mechanism for choline by nerve cells, thereby slowing acetylcholine synthesis. Support for the latter action was provided by Hodgkin and Martin[17] and Schuberth et al.,[18] who demonstrated in in vitro studies with HC-3, inhibition of uptake of isotopically labeled choline into nerve tissues. Choline has been shown to be an effective and specific antagonist[2,3,11] to hemicholinium-induced actions.

About 14 years later, Benz and Long,[19] on reexamination of the original series of compounds of general formula A, became interested in the bis-*meta*-pyridinium analog, where $NR_1R_2R_3$ represents

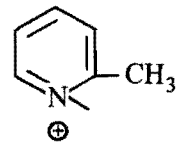


which exhibited an unusually high toxicity of 70 μg/kg in mice, more lethal than HC-3. These investigators evaluated eight substituted pyridinium and N-methylpiperidinium analogs of general structure A for their ability to block neuromuscular transmission and to inhibit human erythrocyte acetylcholinesterase. They concluded that the relatively high lethality of the *meta*-methylpyridinium compound is a result of a dual action mechanism of AChE inhibition and hemicholinium type action.

In the course of structure-activity relationship studies of quaternary ammonium compounds and their mode of action at the peripheral parasympathetic nervous system, some of the substituted pyridinium analogs of general formula A were prepared in our laboratory. Pharma-

[3] Reitzel, N. I., and Long, J. P. J. Pharmacol. Exp. Ther. *127*, 15 (1959).
[4] Marshall, F. N., and Long, J. P. J. Pharmacol. Exp. Ther. *127*, 236 (1959).
[5] Powers, M. F., Kruger, S., and Schueler, F. W. J. Pharm. Sci. *51*, 27 (1962).
[6] Thampi, S. N., Domer, F. R., Haarstad, V. B., and Schueler, F. W. J. Pharm. Sci. *55*, 381 (1966).
[7] Long, J. P., Evans, C. T., and Wong, S. J. Pharmacol. Exp. Ther. *155*, 233 (1966).
[8] DiAugustine, R. P., and Haarstad, V. B. Biochem. Pharmacol. *19*, 559 (1970).
[9] McIntosh, F. C., Birks, R. I., and Sastry, P. B. Nature (London) *178*, 1181 (1956).
[10] McIntosh, F. C. Can. J. Biochem. Physiol. *37*, 343 (1959).
[11] Gardiner, J. E. J. Physiol. (London) *138*, 13P (1957).
[12] Matthews, E. K. Brit. J. Pharmacol. Chemother. *26*, 552 (1966).
[13] McIntosh, F. C. Can. J. Biochem. Physiol. *41*, 2555 (1963).
[14] Bourillet, F. C., and Ogura, Y. Arch. Int. Pharmacodyn. Ther. *139*, 187 (1962).
[15] Evans, E. R., and Wilson, H. Brit. J. Pharmacol. Chemother. *22*, 441 (1964).
[16] Elmquist, D., and Quastel, D. M. J. J. Physiol. (London) *177*, 463 (1965).
[17] Hodgkin, J. E., and Martin, K. J. Physiol. (London) *179*, 26P (1965).
[18] Schuberth, J., Sundwall, A., Sorbo, B., and Lindell, J. O. J. Neurochem. *13*, 347 (1966).
[19] Benz, F. W., and Long, J. P. Pharmacol. Exp. Ther. *166*, 225 (1969).

cological toxicity evaluation confirmed the data reported in literature. However, an additional outstanding characteristic not yet explored was observed in still another member of the series. The *ortho*-pyridinium analog, where $NR_1R_2R_3$ in general formula A represents



showed unusually great safety margins for quaternary ammonium compounds. A ratio between the iv LD50 and iv MED50 of about 300 in mice and 180 in rabbits was found. The compound is equipotent to the *meta*-methylpyridinium isomer, as measured by AChE inhibitions[19] and MED50's, yet 23 times less toxic. These facts appeared to us to be of great interest in respect to cholinergic mechanisms and from the point of view of potential utility in chemotherapy. Compounds with safety margins at least one order of magnitude greater than the presently medicinally employed quaternary ammonium agents (such as curare, decamethonium, and succinylcholine, which are used as muscle relaxants in surgical procedures, and neostigmine, used in myasthenia gravis and other therapeutic applications) could potentially serve as superior drugs in medicine.

II. SYNTHESIS AND EXPERIMENTATION.

A. Synthesis.

In the investigation presented herein, the synthesis of potentially potent compounds affecting the presynaptic biochemical reactions was attempted through elucidation of the structural requirements needed for certain pharmacological effects (see part III). Several series of compounds related to HC-3 (I_1) and its pyridinium analogs were synthesized. They are listed together with their biological data in tables I through VIII. For earlier comparison, those compounds that are more pertinent in the structure-activity relationships were singled out and compiled in table IX.

The final products were synthesized by quaternization of the various tertiary amines, with mono and dihalides as the alkylating agents. In the synthesis of the bis-quaternary compounds schematically shown below, the amines (X) were taken in excess in order to favor completion of the reactions. The mono-quaternary byproducts (XII) were removed utilizing solubility differences. In most instances the mono-quaternary intermediates are soluble in hot acetone, whereas the bis-quaternary products (XIII) are not.

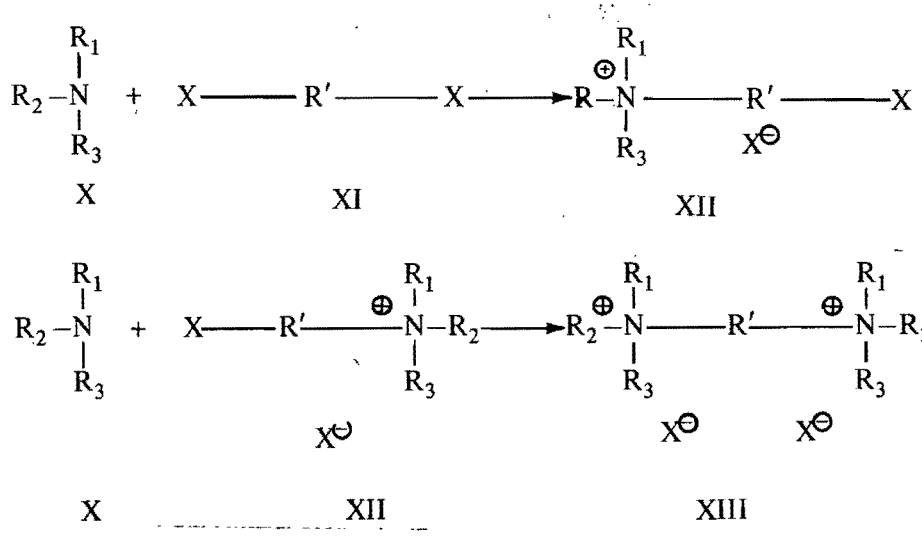
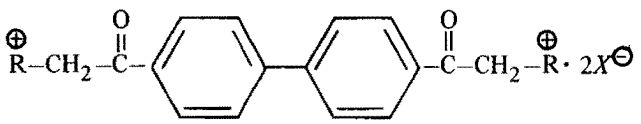
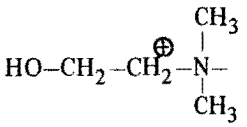
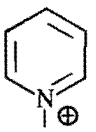
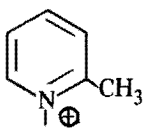
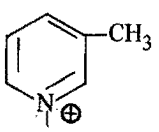
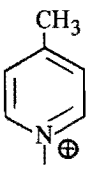
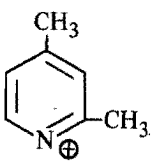


Table I. Biphenacyl Bis-Quaternary Ammonium Compounds

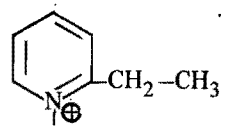
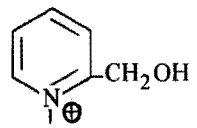
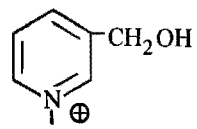
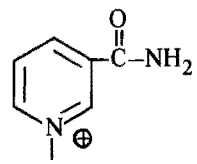
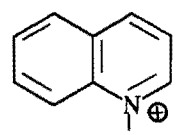
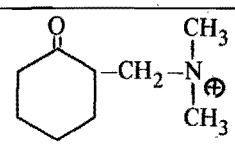
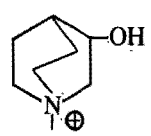
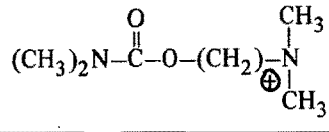


I

Compound No.	EA No.	R	*X*	iv Mice		
				MED50[a]	LD50	LD50/MED50
				mg/kg		
I_1[b]	(HC-3)		Br		0.120[c]	
I_2	4046		I	0.056	1.3	22.4
I_3	4079		Br	0.0056	1.8	320
I_4	4080		Br	0.0056	0.079	14
I_5	4081		Br	0.56	14.0	25
I_6	4919		Br	0.018	1.8	100

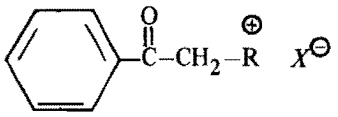
[a-c]See footnotes at end of table.

Table I. Continued

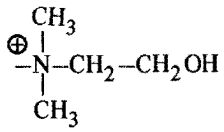
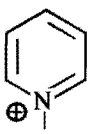
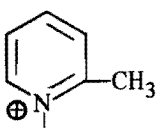
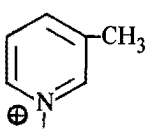
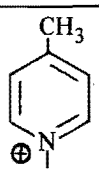
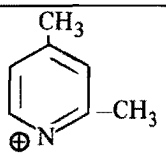
Compound No.	EA No.	R	X	iv Mice		
				MED50[a]	LD50	LD50/MED50
				mg/kg		
I_7	5049		Br	0.18	1.0	5.6
I_8	4720		Br	0.056	1.8	32
I_9	4920		Br	0.042	0.56	13
I_{10}	4050		Br	0.18	1.0	5.6
I_{11}	4881		Br	0.056	1.0	18
I_{12}	3947		Br	0.56	3.16	5.6
I_{13}	3827		Br	0.56	1.0	1.8
I_{14}	3828		Br	1.0	1.78	1.8

[a]The minimal effective dose at which the first pharmacological symptoms appear.
[b]This compound according to IR spectra exists in the cyclized form as the hemiketal,
[c]This value is for the ip LD50. *From* Benz, F. W., and Long, J. P. J. Pharmacol. Exp. Ther. *12*, 15 (1959).

Table II. Phenacyl Mono-Quaternary Ammonium Compounds

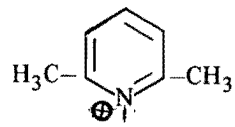
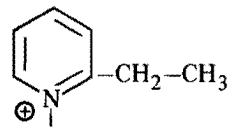
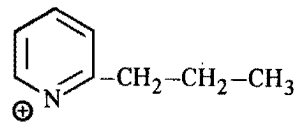
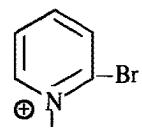
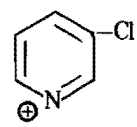
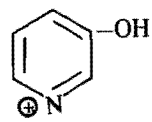


II

Compound No.	EA No.	R	X	iv Mice		
				MED50[a]	LD50	LD50/MED50
				mg/kg		
II_1[b]	3859		$Cl^{\ominus}$	17.8	17.8	1
II_2	4858		$Br^{\ominus}$	18	56	3.2
II_3	4859		$Br^{\ominus}$	5.6	18	3.2
II_4	4885		$Br^{\ominus}$	0.18	5.6	32
II_5	4873		$Br^{\ominus}$	5.6	56	10
II_6	4879		$Br^{\ominus}$	5.6	18	3.2

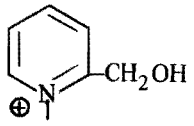
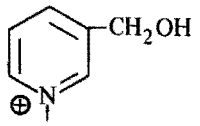
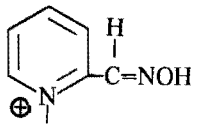
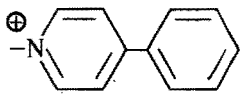
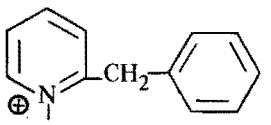
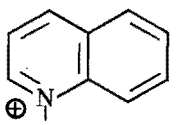
[a,b]See footnotes at end of table.

Table II. Continued

Compound No.	EA No.	R	X	iv Mice		
				MED50[a]	LD50	LD50/MED50
				mg/kg		
II_7	4882		$Br^{\ominus}$	1.8	18	10
II_8	4992		$Br^{\ominus}$	1.8	18	10
II_9	4993		$Br^{\ominus}$	5.6	32	5.6
II_{10}	4789		$Br^{\ominus}$	18	32	1.8
II_{11}	4995		$Br^{\ominus}$	0.18	5.0	28
II_{12}	4996		$Br^{\ominus}$	18	32	1.8

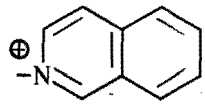
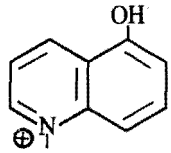
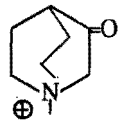
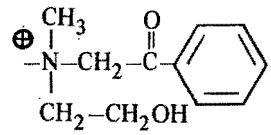
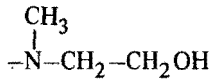
[a]See footnotes at end of table.

Table II. Continued

Compound No.	EA No.	R	X	iv Mice		
				MED50[a]	LD50	LD50/MED50
				mg/kg		
II_{13}	4989		$Br^{\ominus}$	5.6	56	10
II_{14}	4981		$Br^{\ominus}$	5.6	56	10
II_{15}	4883		$Br^{\ominus}$	5.6	18	3.2
II_{16}	5015		$Br^{\ominus}$	5.6	56	10
II_{17}	4997		$Br^{\ominus}$	5.6	56	10
II_{18}	4988		$Br^{\ominus}$	1.8	18	10

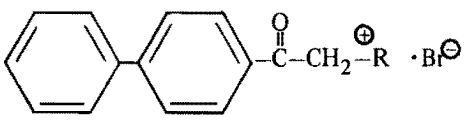
[a]See footnotes at end of table.

Table II. Continued

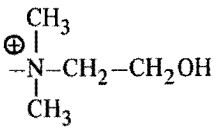
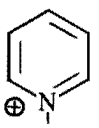
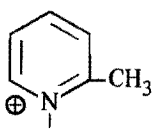
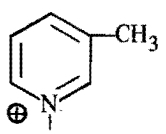
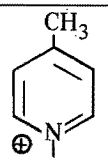
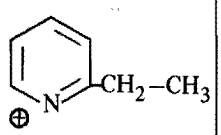
Compound No.	EA No.	R	X	iv Mice		
				MED50[a]	LD50	LD50/MED50
				mg/kg		
II_{19}	5016		$Br^{\ominus}$	5.6	56	10
II_{20}	5020		$Br^{\ominus}$	1.8	>100	>5.6
II_{21}	3861		$Cl^{\ominus}$	17.8	17.8	1
II_{22}	4067		$Cl^{\ominus}$	1.8	14	7.9
II_{23}[b,c]	4082			5.6	>20	>3.6

[a]The minimal effective dose at which the first pharmacological symptoms appear.
[b]This compound according to IR spectra exists in the cyclized form as the hemiketal.
[c]This compound is the tertiary amine.

Table III. Phenyl-Phenacyl Mono-Quaternary Ammonium Compounds

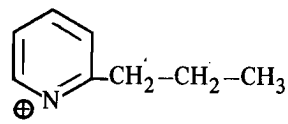
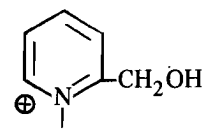
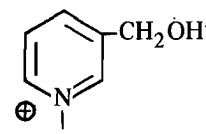
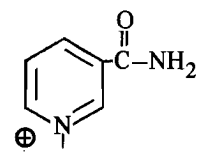
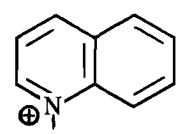
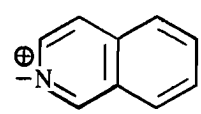


III

Compound No.	EA No.	R	iv Mice		
			MED50[a]	LD50	LD50/MED50
			mg/kg		
III_1[b]	5014		1.8	18	10
III_2	4980		56	>100	1.8
III_3	4977		18	56	3.2
III_4	4978		1.8	5.6	3.2
III_5	4979		18	56	3.2
III_6	4994		5.6	56	10

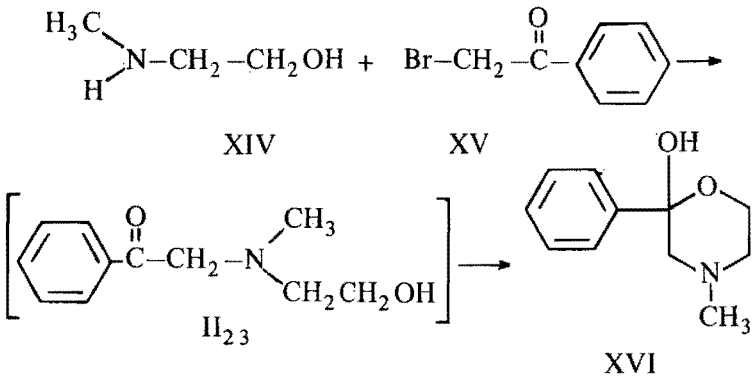
[a,b]See footnotes at end of table.

Table III. Continued

Compound No.	EA No.	R	iv Mice		
			MED50[a]	LD50	LD50/MED50
			mg/kg		
III_7	5094		18	56	3.2
III_8	4984		1.8	32	18
III_9	4985		18	56	3.2
III_{10}	5019		18	18	1
III_{11}	4987		1.8	56	32
III_{12}	5011		5.6	>100	>18

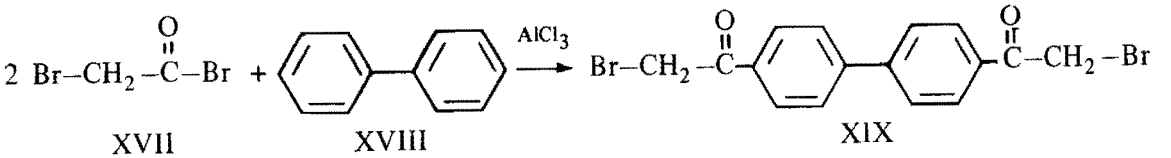
[a]The minimal effective dose at which the first pharmacological symptoms appear.
[b]This compound according to IR spectra exists in the cyclized form as the hemiketal.

The aminocarbamate for the preparation of I_{14} was obtained by carbamylation of the corresponding hydroxy precursor with dimethylcarbamoyl chloride. The tertiary amine II_{23} was prepared by allowing (2-hydroxyethyl)methylamine (XIV) to react with phenacyl bromide (XV). IR spectra proved that cyclization to the hemiketal form of structure XVI occurred.



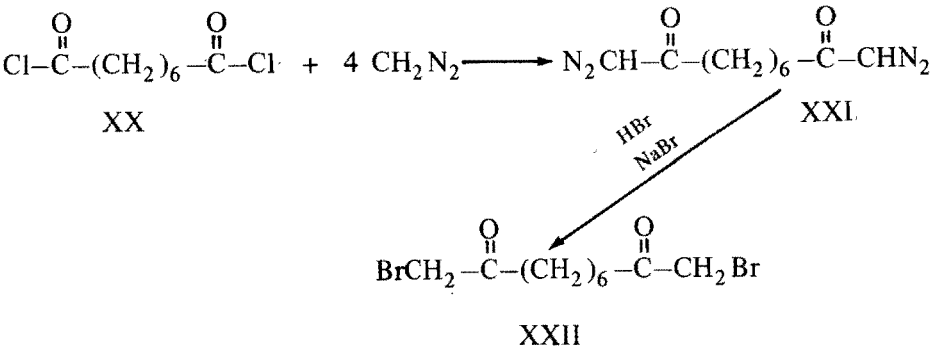
XIV XV II_{23} XVI

The monohalides and the polymethylenedihalides were commercially available. 4,4'-Biphenacyl dibromide (XIX) used in the synthesis of the compounds in table I was prepared by the Friedel-Craft reaction with bromoacetyl bromide (XVII) on biphenyl (XVIII).[1]



XVII XVIII XIX

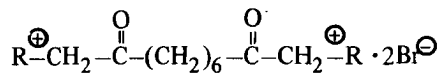
The Arndt-Eistert homologation process[20] was utilized to obtain 1,10-dibromodecane-2,9-dione (XXII), the dihalide for the preparation of the compounds in table IV. Suberoyl chloride (XX) was treated with diazomethane to give the diazonium ketone (XXI), which was decomposed with hydrobromic acid and sodium bromide to give the desired product.



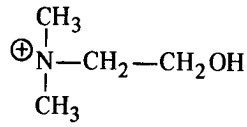
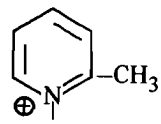
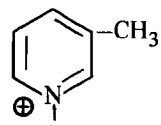
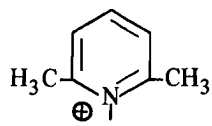
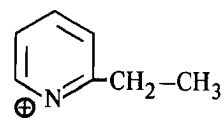
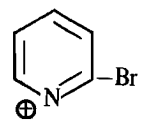
XX XXI XXII

[20] Fahr, E. Ann. der Chemie *638*, 1 (1960).

Table IV. Decamethylene-2,9-Dione Bis-Quaternary Ammonium Compounds

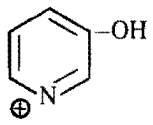
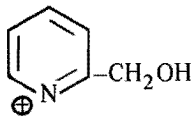
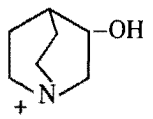
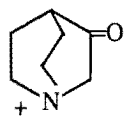


IV

Compound No.	EA No.	R	iv Mice		
			MED50[a]	LD50	LD50/MED50
			mg/kg		
IV_1[b,c]	4075		0.18	1.0	5.6
IV_2	4684		0.18	1.0	5.6
IV_3[c]	4076		0.056	1.0	18
IV_4	4718		0.18	1.0	5.6
IV_5	4913		0.56	1.8	3.2
IV_6	4691		0.56	5.6	10

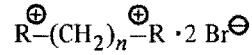
[a-c]See footnotes at end of table.

Table IV. Continued

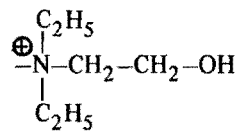
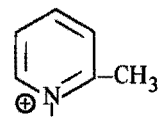
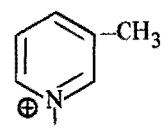
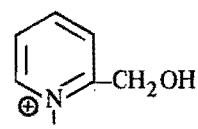
Compound No.	EA No.	R	iv Mice		
			MED50[a]	LD50	LD50/MED50
			mg/kg		
IV_7	4833		18	>100	>5.6
IV_8	4831		0.56	18	32
IV_9	4719		0.18	1.8	10
IV_{10}	4832		5.6	10	1.8

[a]The minimal effective dose at which the first pharmacological symptoms appear.
[b]This compound according to IR spectra exists in the cyclized form as the hemiketal.
[c]This compound was isolated as the iodide salt.

Table V. Polymethylene Bis-Quaternary Ammonium Compounds

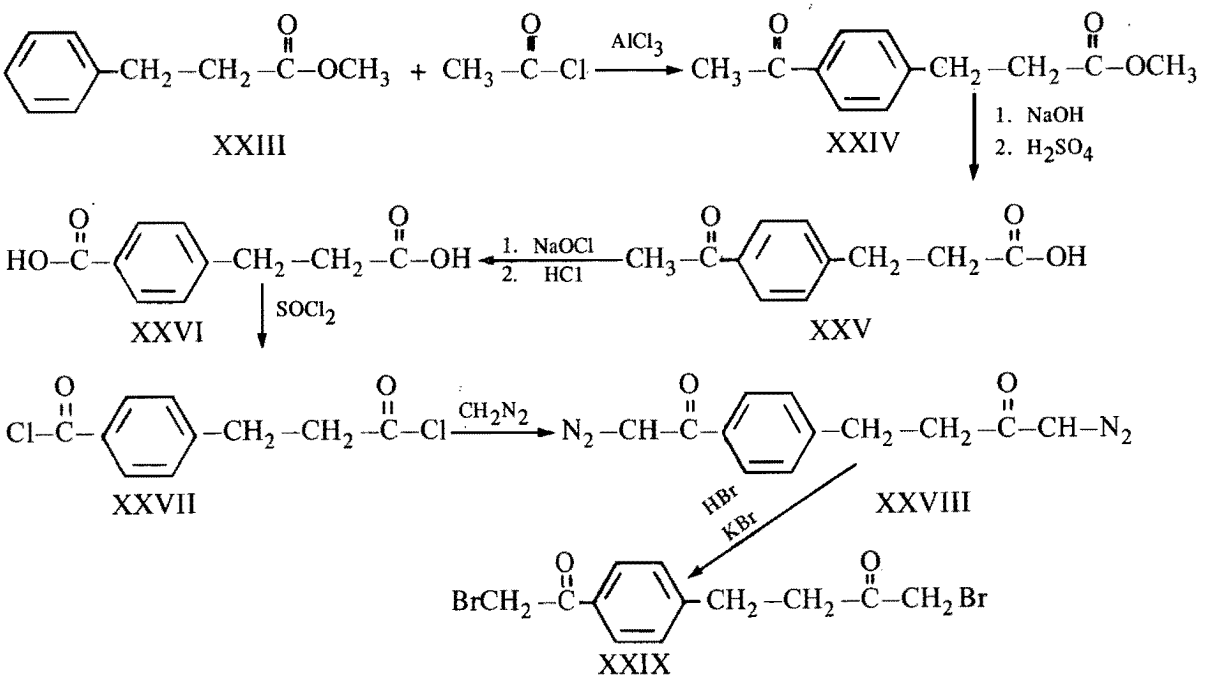


V

Compound No.	EA No.	R	*n*	iv Mice		
				MED50*	LD50	LD50/MED50
				mg/kg		
V_1	3831		10	0.56	1.78	3.17
V_2	5199		8	1.8	10	5.6
V_3	5147		8	0.56	5.6	10
V_4	3958		10	0.56	17.8	31.7

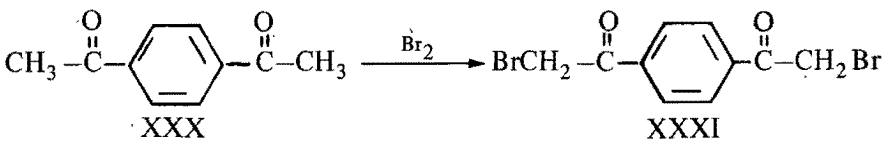
*The minimal effective dose at which the first pharmacological symptoms appear.

4-[*p*-(Bromoacetyl)phenyl]-1-bromo-2-butanone (XXIX),[21] the dihalide for the synthesis of the compounds in table VI, was prepared in six steps. A Friedel-Craft reaction with acetyl chloride on methyl β-phenylpropionate (XXIII) produced methyl β-(*p*-acetylphenyl) propionate (XXIV), which after basic hydrolysis gave β-(*p*-acetylphenyl)propionic acid (XXV). Oxidation with sodium hypochlorite provided the dicarboxylic acid (XXVI), which was converted to its diacid chloride (XXVII) with thionyl chloride and subjected to the Arndt-Eistert homologation process to form the diazoketone (XXVIII). Decomposition with hydrobromic acid and sodium bromide led to the final product (XXIX).



XXIII XXIV XXV XXVI XXVII XXVIII XXIX

Bromination of 1,4-diacetylbenzene (XXX) in acetic acid produced 1,4-bis(bromoacetyl)benzene[22] (XXXI) in excellent yield for the synthesis of the analogs in table VII.

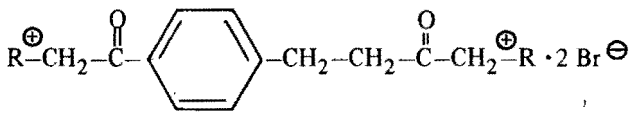


XXX XXXI

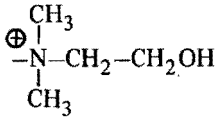
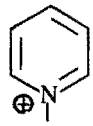
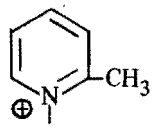
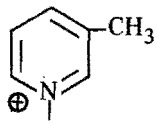
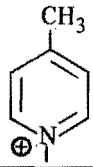
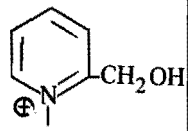
The unsymmetrical bis-quaternary ammonium compounds listed in table VIII were synthesized by alkylation of the corresponding tertiary amines with the mono-quaternary halides (XXXII) and (XXXIII). The mono-quaternary halides were obtained by allowing an excess of the dihalides (XIX) and (XXIX) to react with *ortho*-methylpyridine.

[21] Braddeley, G., and Williamson, R. J. Chem. Soc. 4650 (1956).
[22] Krohnke, F., and Vogt, I. Chem. Ber. *86*, 1136 (1953).

Table VI. Phenacyl Butanone Bis-Quaternary Ammonium Compounds



VI

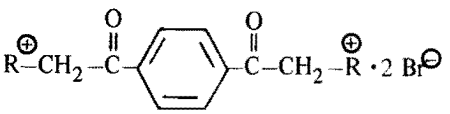
Compound No.	EA No.	R	iv Mice		
			MED50[a]	LD50	LD50/MED50
			mg/kg		
VI_1[b]	5236		0.018	0.090[c]	5
VI_2	5298		0.18	5.6	32
VI_3	5195		0.0056	0.56	100
VI_4	5244		0.018	0.28	16
VI_5	5245		1.8	18	10
VI_6	5243		0.18	1	5.6

[a]The minimal effective dose at which the first pharmacological symptoms appear.

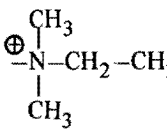
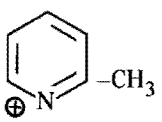
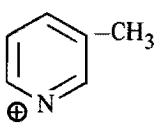
[b]This compound according to IR spectra exists in the cyclized form as the hemiketal.

[c]The ip LD50 was found to be 0.059 mg/kg.

Table VII. Phenylenediacetyl Bis-Quaternary Ammonium Compounds

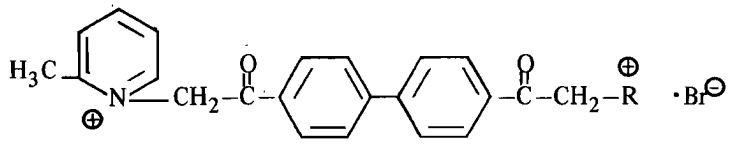


VII

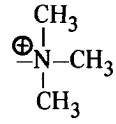
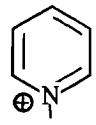
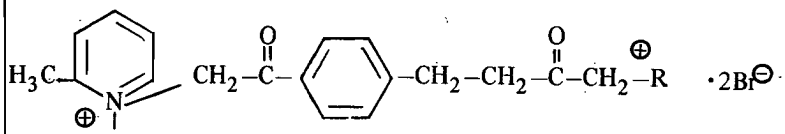
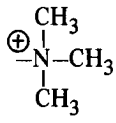
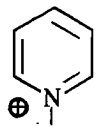
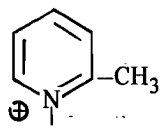
Compound No.	EA No.	R	iv Mice		
			MED50[a]	LD50	LD50/MED50
			mg/kg		
VII_1[b]	5371		0.056	0.63	11
VII_2	5382		0.056	5.6	100
VII_3	5299		0.018	0.14	8

[a]The minimal effective dose at which the first pharmacological symptoms appear.
[b]This compound according to IR spectra exists in the cyclized form as the hemiketal.

Table VIII. Unsymmetrical Bis-Quaternary Ammonium Compounds

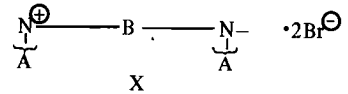


VIII

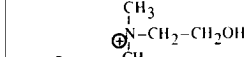
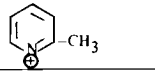
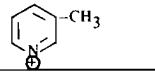
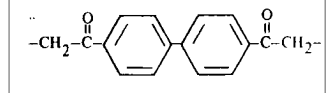
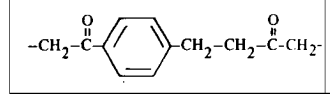
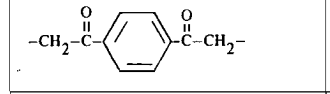
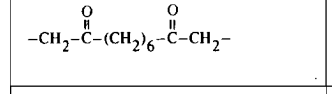
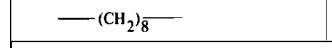
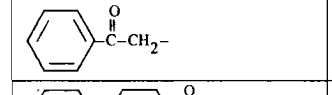
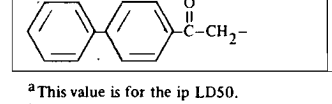
Compound No.	EA No.	R	iv Mice		
			MED50*	LD50	LD50/MED50
			mg/kg		
$VIII_1$	5490		0.56	2.8	5
$VIII_2$	5448		0.056	0.5	8.9
IX					
IX_1	5352		0.18	1.6	8.9
IX_2	5345		0.18	0.56	
IX_3	5398		0.056	5.6	100

*The minimal effective dose at which the first pharmacological symptoms appear.

Table IX. *Ortho*-Pyridium, *Meta*-Pyridium, and Dimethylethanol Ammonium Compounds

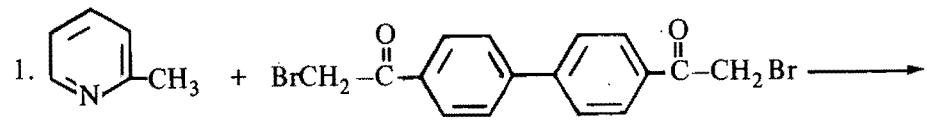


X

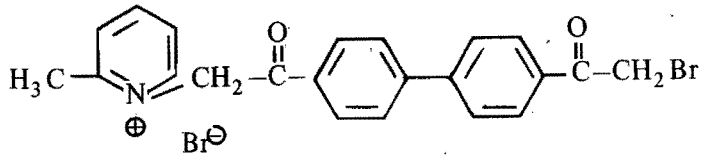
B Chains connecting onium centers	A, Onium centers											
												
	Compound No.	iv Mice			Compound No.	iv Mice			Compound No.	iv Mice		
		MED50	LD50	LD50/MED50		MED50	LD50	LD50/MED50		MED50	LD50	LD50/MED50
		mg/kg				*mg/kg*				*mg/kg*		
	I_1		0.120[a]		I_3	0.0056	1.8	320	I_4	0.0056	0.079	14
	VI_1	0.018	0.90[b]	5	VI_3	0.0056	0.56	100	VI_4	0.018	0.28	16
	VII_1	0.056	0.63	11	VII_2	0.056	5.6	100	VII_3	0.018	0.14	8
	IV_1	0.18	1.0	5.6	IV_2	0.18	1.0	5.6	IV_3	0.056	1.0	18
					V_2	1.8	1.0	5.6	V_3	0.56	5.6	10
 XI												
	II_1	17.8	17.8	1	II_3	5.6	18	3.2	II_4	0.18	5.6	32
	III_1	1.8	18	10	III_3	18	56	3.2	III_4	1.8	5.6	3.2

[a] This value is for the ip LD50.

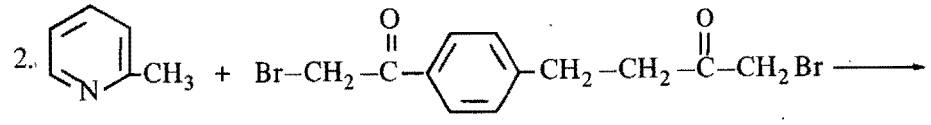
[b] The ip LD50 was found to be 0.059 mg/kg.



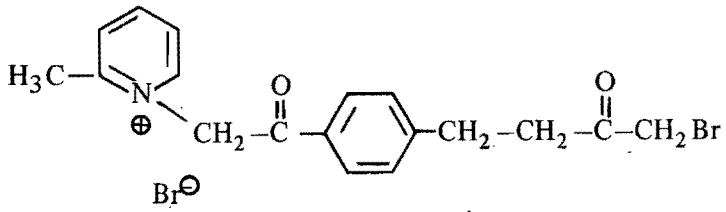
XIX



XXXII



XXIX



XXXIII

B. Amines.

1. (3-Dimethylcarbamoylpropyl)Dimethylamine.

A solution of 31 grams (0.30 mole) of 3-dimethylamino-1-propanol and 15 grams (0.14 mole) of dimethylcarbamoyl chloride in 200 ml of benzene was refluxed for 18 hours on a steam bath. On cooling a solid formed, which was separated from the reaction mixture by filtration. The filtrate was distilled at atmospheric pressure to remove the solvent, and the remaining liquid was distilled at reduced pressure. Collected were 21.1 grams (86% yield) of material boiling at 155° to 157°C at 70 mm pressure, n_D^{27} 1.4395.

2. N-Phenacyl-N-(2-Hydroxyethyl)-N-Methylamine, EA 4082 (II_{23}).

A solution of 15.9 grams (0.08 mole) of phenacyl bromide and 18.0 grams (0.24 mole) of 2-methylaminoethanol in 100 ml of benzene was stirred for 2 hours at room temperature. An oil, which settled at the bottom, was separated. The benzene layer was washed with two 25-ml portions of water and then evaporated under reduced pressure on a steam bath. The residue was distilled at 175° to 178°C at 20 mm pressure to give 12.3 grams (80% yield) of product, n_D^{23} 1.5470.

Anal of $C_{11}H_{15}NO_2$: Calculated: C, 68.4; H, 7.8; O, 16.5. Found: C, 68.6; H, 7.7; O, 16.0.

C. Dihalides.

1. 4.4'-Bis(Bromoacetyl)Biphenyl (XIX).

A mixture of 690 ml of carbon bisulfide, 116.0 grams (0.75 mole) of biphenyl, and 277.0 grams (2.08 moles) of anhydrous aluminum chloride was placed in a 2-liter, three-neck flask fitted with a thermometer, stirrer, condenser, dropping funnel, Drierite tube, and hydrogen bromide trap. From the dropping funnel, 369.0 grams (1.83 moles) of bromoacetyl bromide were added to the stirred mixture over a period of 2 hours. During this time, hydrogen bromide was evolved and absorbed in the trap. The reaction mixture was stirred and refluxed for 2 hours, and at the end of this time no more hydrogen bromide was evolved. The content of the flask was poured into 1 liter of stirred concentrated hydrochloric acid in water, and the mixture was passed through a filter. The solid on the filter was washed and air dried to give 266.2 grams of crude 4,4'-bis(bromoacetyl)biphenyl. This material was treated with 4500 ml of boiling tetrahydrofuran; the small amount of insoluble material was filtered off, and the filtrate was carbon-treated twice and filtered. The filtrate was concentrated atmospherically until solid appeared. At this point heat was removed, and the flask and its contents were cooled in an ice water bath. The solid that formed was collected on a filter and washed with cold tetrahydrofuran to give 100.0 grams of product. The filtrate was concentrated to approximately one-fourth of its original volume to give an additional 15.0 grams of product; 115 grams (40.6% yield) of product melting at 223° to 225°C were obtained.

2. 1,10-Bis(Diazo)Decane-2,9-Dione (XXI).

A 2-liter, three-neck flask (flask A) was equipped with a mechanical stirrer using a rubber bearing, a liquid inlet tube, and a distillation head leading via a condenser to a 5-liter, three-neck flask (flask B) equipped with a mechanical stirrer with a rubber bearing, a liquid inlet tube, and a reflux condenser leading to a vent line. Flask A was placed in an oil bath, and the temperature was automatically controlled at 55° to 60°C. Flask B was placed in a refrigerated bath at - 15° to - 10°C. The entire apparatus was set up in a steel barricade room. A feed line was connected from each of the inlet tubes of the apparatus to a Sigmamotor metering pump located outside the barricade room. The actual runs were conducted in the following manner.

Flask A was charged with 90 ml of water, 83 grams of 85% potassium hydroxide, and 425 ml of 2-(2-ethoxyethoxy)ethanol; flask B was charged with 150 ml of ether. After the constant temperature baths had reached equilibrium, ether was pumped into flask A at the rate of 10 ml per minute until distillation started. A Diazald solution (250 grams of Diazald, Aldrich Chemical Company, made to 1800 ml with ether) then was substituted for the plain ether and metered in at a rate of 6 ml per minute. When 600 ml of the Diazald solution had been added, a solution of 42 grams of suberoyl dichloride diluted to 250 ml with ether was metered into flask B at the rate of 0.83 ml per minute until the addition of Diazald solution to flask A was completed, after which time the rate was increased to 2.2 ml per minute. Each feed line was flushed with about 100 ml of ether after the respective additions were completed. The reaction mixture was stirred for 30 minutes after the suberoyl chloride solution addition was completed; then it was sparged with nitrogen for another 30-minute period to remove as much excess diazomethane as possible. The yellow solid was filtered, washed with ether, and dried in vacuo. The crude diazo compound was recrystallized from 100 ml of carbon tetrachloride to yield an average of 21.5 grams of 1,10-bis(diazo) decane-2,9-dione (melting at 77° to 79°C) per run for seven runs.

3. 1,10-Dibromodecane-2,9-Dione (XXII).

To a 5-liter, three-neck flask fitted with a mechanical stirrer and dropping funnel, 270 grams (2.27 moles) of potassium bromide and 306 grams (1.80 moles) of 48% hydrobromic acid in 1310 ml of water were added. The solution was cooled to 5°C in an ice bath, and a solution of 50 grams (0.23 mole) of 1,10-bis(diazo)decane-2,9-dione in 1300 ml of methanol was added dropwise

over 90 minutes while the temperature was maintained between 5° and 10°C. A white solid precipitated immediately. After the addition was completed, the reaction mixture was stirred for 15 minutes, diluted with 1100 ml of water, and stirred for several minutes. The solids were collected by filtration of the cold slurry and dried to constant weight over phosphorus pentoxide under vacuum. The crude product was recrystallized from methanol to give 59 grams (70.3% yield) of 1,10-dibromodecane-2,9-dione, obtained as off-white plates that melted at 96° to 98°C.

4. Methyl β-(4-Acetylphenyl)Propionate (XIX).

A 2-liter, three-neck flask fitted with a stirrer, thermometer, condenser, Drierite tube, addition funnel, and HCl trap was charged with 332 grams (2.49 moles) of aluminum chloride, 332 grams (4.26 moles) of acetyl chloride, and 650 ml of methylene chloride. A solution of 150 grams (0.913 mole) of methyl β-phenylpropionate in 330 ml of methylene chloride was added dropwise to the reaction flask. When the reaction was completed, the mixture was poured into ice and hydrochloric acid and extracted with ether. The extracts were combined, dried with magnesium sulfate, filtered, and concentrated under vacuum. The residue was distilled through a short Vigreux column to give 140.2 grams (74.8% yield) of methyl β-(4-acetylphenyl)propionate, $b_{2.5}$ 148° to 149.5°C, n_D^{25} 1.5246.

5. β-(4-Acetylphenyl)Propionic Acid (XXV).

Methyl β-(4-acetylphenyl)propionate, 335.5 grams (1.628 moles), was added to a solution of 216.0 grams of sodium hydroxide in 860 ml of water, and the mixture was stirred and refluxed for 3 hours. Then 400 ml of water were distilled off, the residue was diluted with 800 ml of water, and an additional 400 ml of water were removed. The residue was cooled and acidified with dilute sulfuric acid. The precipitated β-(4-acetylphenyl)propionic acid was filtered off, washed with cold water, and purified by repeated extraction with hot water to give 119.0 grams (34.8% yield) of β-(4-acetylphenyl)-propionic acid, which melted at 118° to 120°C.

6. β-(4-Carboxyphenyl)Propionic Acid (XXVI).

Chlorine, 120.8 grams (1.701 moles), was added to a mixture of 163.5 grams (4.087 moles) of sodium hydroxide, 225 ml of water, and 940 grams of ice in a 3-liter, three-neck flask. β-(4-Acetylphenyl)propionic acid, 60 grams (0.31 mole), was dissolved in a solution of 48.8 grams (0.871 mole) of potassium hydroxide in 400 ml of water and added dropwise with stirring to the sodium hypochlorite solution. The temperature rose to 37°C during the addition, and stirring was continued for several hours until the temperature dropped to 25°C. Sodium sulfite, 77.0 grams, was added to decompose excess sodium hypochlorite, and the solution was acidified with concentrated hydrochloric acid. The solid that formed was filtered, washed thoroughly with water, and dried. The β-(4-carboxyphenyl)propionic acid weighed 55.0 grams (91.6% yield) and melted at 280° to 283°C.

7. β-(4-Chloroformylphenyl)Propionyl Chloride (XXVII).

A mixture of 60 grams (0.31 mole) of β-(4-carboxyphenyl)propionic acid and 360 ml of thionyl chloride was stirred at reflux temperature for 12½ hours. The excess thionyl chloride was removed under vacuum, and the residue was distilled through a short Vigreux column to give 55.3 grams (76.4% yield) of β-(4-chloroformylphenyl)propionyl chloride, $b_{0.5}$ 126°C, n_D^{25} 1.5645. This material analyzed 97.7% by saponification with sodium hydroxide.

8. 4-(4-Diazoacetylphenyl)-1-Diazo-2-Butanone (XXVIII).

This intermediate was prepared using the apparatus and procedures described for 1,10-bis(diazo)decane-2,9-dione (XXV). A run using 55 grams (0.24 mole) of β-(4-chloroformylphenyl)propionyl chloride and the diazomethane obtained from 300 grams of Diazald gave

43 grams of the crude diazodiketone. The crude product was recrystallized from 1000 ml of carbon tetrachloride to yield 40 grams of 4-(4-diazoacetylphenyl)-1-diazo-2-butanone melting at 82° to 84°C.

9. 4-[4-(Bromoacetyl)Phenyl]-1-Bromo-2-Butanone (XXIX).

4-(4-Diazoacetylphenyl)-1-diazo-2-butanone, 39.2 grams (0.162 mole), was dissolved in 950 ml of methanol; this solution was added over a period of 3 hours to a solution of 196.8 grams of potassium bromide and 222.4 grams of 48% hydrobromic acid in 960 ml of water, while the temperature was kept at 5° to 10°C. When the addition was completed, the reaction mixture was stirred for 1 hour and diluted with 700 ml of water. The solid that formed was collected on a filter, washed several times with cold water, vacuum-dried over phosphorus pentoxide, and recrystallized from methanol to give 36.0 grams (64.7% yield) of 4-[4-(bromoacetyl)phenyl]-1-bromo-2-butanone, which melted at 104.5° to 106.5°C.

10. 1,4-Bis(Bromoacetyl)Benzene (XXXI).

To a 1-liter flask containing 25 grams (0.15 mole) of *p*-diacetylbenzene in 500 ml of glacial acetic acid warmed to 50°C was added, with continuous stirring, 50 grams (0.31 mole) of bromine in 50 ml of glacial acetic acid over a half-hour period. During the addition, the temperature was raised to 75°C. Hydrogen bromide gas evolved. After the addition, the mixture was stirred for 2 hours at 70° to 75°C and then allowed to stand overnight at room temperature. The solid that had formed was collected to give 37 grams of crude green material. Recrystallization from ethanol resulted in a total of 24 grams (50% yield) of 1,4-bis(bromoacetyl)benzene, mp 171° to 173°C.

D. Mono-Quaternary Halides.

1. 4-(2-Methylpyridinio)Acetyl-4'-Bromoacetylbiphenyl Bromide (XXXII).

4,4'-Bis(bromoacetyl)biphenyl, 19.8 grams (0.05 mole), was dissolved in 600 ml of hot tetrahydrofurane, and 2.3 grams (0.025 mole) of 2-methylpyridine were added. The solution was refluxed for 4 hours, during which time a solid formed. The hot reaction mixture was passed through a filter, and the collected solid was recrystallized from water to give 6.2 grams (50.8% yield) of product. The compound melted with decomposition at 227° to 229°C.

Anal of $C_{22}H_{19}Br_2NO_2$: Calculated: C, 54.0; H, 3.9; N, 2.9. Found: C, 54.0; H, 4.1; N, 2.8.

2. 4-[4-(2-Methylpyridinioacetyl)Phenyl]-1-Bromo-2-Butanone Bromide (XXXIII).

A solution of 35 grams (0.1 mole) of 4-[4-(bromoacetyl)phenyl]-1-bromo-2-butanone and 4.6 grams (0.05 mole) of 2-methylpyridine in 500 ml of ether and 30 ml of acetone was allowed to stand at room temperature overnight. The precipitate that formed was collected on a filter, washed with acetone, and stirred in approximately 500 ml of acetonitrile. The undissolved bis-quaternary byproduct was removed by filtration, and the filtrate was removed by distillation. The crude product thus obtained was purified by dissolving it in methanol and subsequently precipitating it with ethyl acetate. The pure product, 4.5 grams (20.5% yield), obtained melted with decomposition at 170° to 173°C.

Anal of $C_{18}H_{19}Br_2NO_2$: Calculated: C, 49.0; H, 4.4; Br, 36.3; N, 3.2. Found: C, 49.0; H, 4.4; Br, 36.0; N, 3.2.

E. Quaternary Ammonium Compounds.

The quaternization was performed generally by heating a solution of the tertiary amine and the halide in an appropriate solvent under reflux conditions or allowing the reactants to stand at room temperature. The product either precipitated directly from the reaction mixture or after addition of relatively nonpolar solvents, such as ethyl acetate and ether. After filtration and drying, the product was obtained, in some instances, in analytically pure form. In other instances, the crude product was purified by recrystallization or by treatment with activated charcoal. In the synthesis of the bis-quaternary ammonium compounds, excesses of the amines were used to minimize the mono-quaternary byproduct that formed during the course of the reaction. The pertinent physical data, yields, and elemental analyses are given in tables X, XI, and XII. Five representative examples follow.

1. Mono-Quaternary Halides.

a. 1-Phenacyl-3-Methylpyridinium Bromide, EA 4885 (II_4).

Phenacyl bromide, 2 grams (0.01 mole), was added in portions to 10 ml of 3-methylpyridine at room temperature, and the reaction mixture was left at room temperature for 1 hour. Acetone was then added, and the reaction mixture was passed through a filter. The solid material on the filter was dissolved in ethanol, treated with activated charcoal, and precipitated with ethyl acetate. The product, 2.1 grams (67% yield), thus obtained melted at 189° to 191°C.

Anal of $C_{14}H_{14}BrNO$: Calculated: C, 57.5; H, 4.8; Br, 27.3; N, 4.8. Found: C, 57.7; H, 4.7; Br, 27.2; N, 4.8.

b. N-(4-Phenylphenacyl)Quinolinium Bromide, EA 4987 (III_{11}).

A solution of 1.4 grams (0.005 mole) of 4-phenylphenacyl bromide and 1.3 grams (0.01 mole) of quinoline in 20 ml of acetone was left at room temperature overnight. The solid that formed was collected on a filter, washed with ethyl acetate, and dried under vacuum to give yellow needle-like analytically pure crystals, 1.3 grams (62% yield), m.p. 229° to 230°C.

Anal for $C_{23}H_{18}BrNO$: Calculated: C, 68.3; H, 4.5; Br, 19.8. Found: C, 68.5; H, 4.8; Br, 19.8.

2. Bis-Quaternary Dihalides.

a. α,ω-Bis(2-Hydroxymethylpyridinio)-4-(4-Acetylphenyl)-2-Butanone Dibromide, EA 5243 (VI_6).

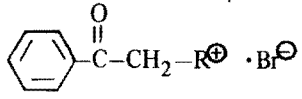
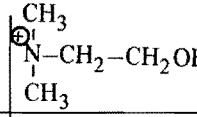
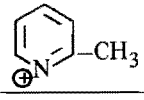
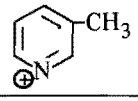
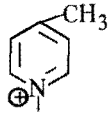
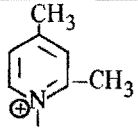
A solution of 1.4 grams (0.004 mole) of 4[4-(bromoacetyl)phenyl]-1-bromo-2-butanone and 1.9 grams (0.017 mole) of 2-hydroxymethylpyridine in 10 ml of acetonitrile was allowed to stand at room temperature overnight. The solid that precipitated was separated by filtration and recrystallized from 50 ml of methanol; 2.1 grams (95% yield) of product, mp 251° to 252°C, were obtained.

Anal for $C_{24}H_{26}Br_6N_2O_4$: Calculated: C, 50.9; H, 4.6; Br, 28.2; N, 4.9; O, 11.3. Found: C, 51.1; H, 4.8; Br, 28.1; N, 5.0; O, 11.6.

b. 1,10-Bis(2-Methylpyridinio)Decanedione-2,9-Dibromide, EA 4684 (IV_2)

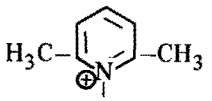
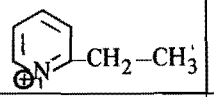
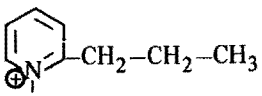
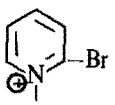
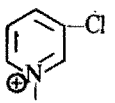
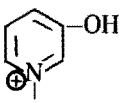
A solution of 0.94 grams (0.01 mole) of 2-methylpyridine and 1.3 grams (0.004 mole) of 1,10-dibromo-2,9-decanedione in 20 ml of acetonitrile was allowed to stand at room temperature

Table X. Physical and Analytical Data of Mono-Quaternary Ammonium Compounds

Compound No.	EA No.	R	Reaction solvent	Empirical formula	Mol wt	Yield	mp[a]	Anal					
								Calcd			Found		
								C	H	Br	C	H	Br
					gm	*%*	*°C*						
													
II_1[b,c]	3859		Acetone	$C_{12}H_{18}ClNO_2$	243	79	199-200	59.1	7.4	14.6	58.9	7.4	14.5
$\frac{II_2}{II}$	4858		Acetone	$C_{13}H_{12}BrNO$	278	68	199-200	56.1	4.3	28.7	56.0	4.3	28.5
II_3	4859		Acetone	$C_{14}H_{14}BrNO$	292	67	219-221	57.6	4.8	27.3	57.7	4.7	27.2
II_4	4885		3-Methyl pyridine	$C_{14}H_{14}BrNO$	292	67	189-191	57.6	4.8	27.3	57.7	4.7	27.2
II_5	4873		4-Methyl pyridine	$C_{14}H_{14}BrNO$	292	77	269-271	57.6	4.8	27.3	57.6	4.7	27.3
II_6	4879		2,4-Lutidine	$C_{15}H_{16}BrNO$	306	71	218-220	58.8	5.2	26.1	58.9	5.3	26.1

[a-c]See footnotes at end of table.

Table X. Continued

Compound No.	EA No.	R	Reaction solvent	Empirical formula	Mol wt	Yield	mp[a]	Anal					
								Calcd			Found		
								C	H	Br	C	H	Br
					gm	%	°C						
II_7	4882		2,6-Lutidine	$C_{15}H_{16}BrNO$	306	33	172-174	58.8	5.2	26.1	59.0	5.4	26.1
II_8	4992		Acetone	$C_{15}H_{16}BrNO$	306	100	186-187	58.8	5.2	26.1	59.0	5.1	26.2
II_9	4993		Acetone	$C_{16}H_{18}BrNO$	320	89	149-150	60.0	5.6	25.0	59.9	5.9	24.9
II_{10}	4789		2-Bromo-pyridine	$C_{13}H_{11}Br_2NO$	357	59	177-180	43.7	3.1	44.8	43.5	3.5	44.4
II_{11}	4995		Acetone	$C_{13}H_{11}BrClNO$	312	94	179-181	49.9	3.5	25.6	49.8	3.7	25.5
II_{12}	4996		Acetone	$C_{13}H_{12}BrNO_2$	294	90	225-226	53.1	4.1	27.2	53.2	4.0	26.9

[a]See footnotes at end of table.

Table X. Continued

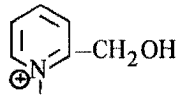
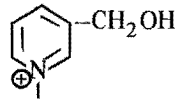
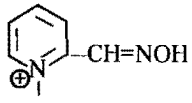
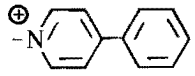
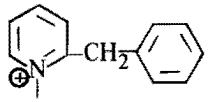
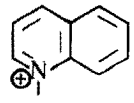
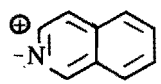
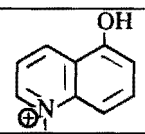
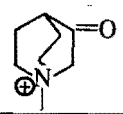
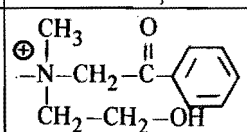
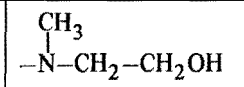
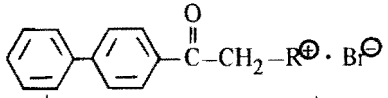
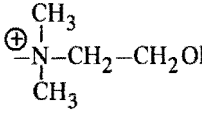
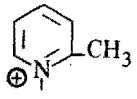
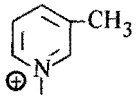
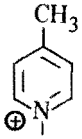
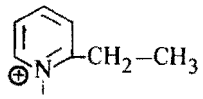
Compound No.	EA No.	R	Reaction solvent	Empirical formula	Mol wt	Yield	mp[a]	Anal					
								Calcd			Found		
								C	H	Br	C	H	Br
					gm	*%*	*°C*						
II_{13}	4989		Acetone	$C_{14}H_{14}BrNO_2$	308	84	190-192	54.5	4.6	26.0	54.7	4.5	25.8
II_{14}	4981		Acetone	$C_{14}H_{14}BrNO_2$	308	100	187-189	54.5	4.6	26.0	54.6	4.8	25.7
II_{15}	4883		Acetone	$C_{14}H_{13}BrN_2O_2$	321	36	197-199	52.3	4.1	24.9	52.0	4.1	24.8
II_{16}	5015		Acetone	$C_{19}H_{16}BrNO$	354	91	244-245	64.4	4.5	22.6	64.7	4.7	22.4
II_{17}	4997		Acetone	$C_{20}H_{18}BrNO$	368	89	181-182	65.2	4.9	21.7	65.4	4.8	21.6
II_{18}	4988		Quinoline	$C_{17}H_{14}BrNO$	328	82	191-192	62.2	4.3	24.3	62.3	4.5	24.2

Table X. Continued

Compound No.	EA No.	R	Reaction solvent	Empirical formula	Mol wt	Yield	mp[a]	Anal					
								Calcd			Found		
								C	H	Br	C	H	Br
					gm	*%*	*°C*						
II_{19}	5016		Isoquinoline	$C_{17}H_{14}BrNO$	328	100	208-209	62.2	4.3	24.3	62.4	4.6	24.2
II_{20}	5020		Methanol	$C_{17}H_{14}BrNO_2$	344	66	260-262	59.3	4.1	23.3	59.7	4.1	23.8
II_{21}[c]	3861		Acetone	$C_{15}H_{18}ClNO_2$	280	82	214-216	64.4	6.4	12.7	64.3	6.5	12.7
II_{22}[c]	4067		Ethanol	$C_{19}H_{22}ClNO_3$	347	31	190-191	65.7	6.3	10.1	65.6	6.4	10.1
II_{23}[d]	4082		Benzene	$C_{11}H_{15}NO_2$	193	80	175-178/ 20 mm	68.4	7.8		68.6	7.7	

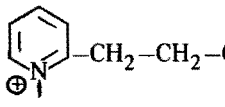
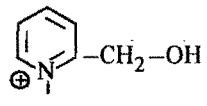
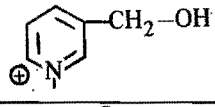
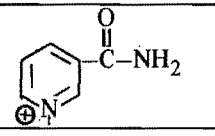
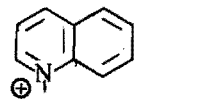
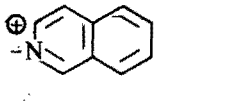
[a,c,d]See footnotes at end of table.

Table X. Continued

Compound No.	EA No.	R	Reaction solvent	Empirical formula	Mol wt	Yield	mp[a]	Anal					
								Calcd			Found		
								C	H	Br	C	H	Br
					gm	%	°C						
													
III_1[b]	5014		Acetone	$C_{18}H_{22}BrNO_2$	364	80	186-187	59.3	6.0	22.0	59.4	5.9	21.8
III_2	4980		Pyridine	$C_{19}H_{16}BrNO$	354	94	227-228	64.4	4.5	22.6	64.3	4.8	22.5
III_3	4977		2-Methyl-pyridine	$C_{20}H_{18}BrNO$	368	89	198-199	65.2	4.9	21.7	65.0	4.9	21.4
III_4	4978		3-Methyl-pyridine	$C_{20}H_{18}BrNO$	368	98	199-200	65.2	4.9	21.7	65.1	5.1	21.6
III_5	4979		4-Methyl-pyridine	$C_{20}H_{18}BrNO$	368	92	260-261	65.2	4.9	21.7	65.4	4.9	21.7
III_6	4994		Acetone	$C_{21}H_{20}BrNO$	382	92	266-267	66.0	5.3	20.9	65.8	5.3	20.8

[a,b]See footnotes at end of table.

Table X. Continued

Compound No.	EA No.	R	Reaction solvent	Empirical formula	Mol wt	Yield	mp[a]	Anal					
								Calcd			Found		
								C	H	Br	C	H	Br
					gm	%	°C						
III_7	5094		Acetone	$C_{22}H_{22}BrNO \cdot 2/3H_2O$ (2.94% H_2O)	408	73	169-170	64.6	6.0	19.5	64.5	5.8	19.6
III_8	4984		Acetone	$C_{20}H_{18}BrNO_2$	384	70	201-202	62.5	4.7	20.8	62.7	4.8	20.7
III_9	4985		Acetone	$C_{20}H_{18}BrNO_2$	384	82	210-211	62.5	4.7	20.8	62.5	4.9	20.5
III_{10}	5019		Acetone	$C_{20}H_{17}BrN_2O_2$	397	72	246-247	60.5	4.3	20.2	60.3	4.3	19.9
III_{11}	4987		Acetone	$C_{23}H_{18}BrNO$	404	62	229-230	68.3	4.5	19.8	68.5	4.8	19.8
III_{12}	5011		Acetone	$C_{23}H_{18}BrNO$	404	90	229-230	68.3	4.5	19.8	68.1	4.4	19.6

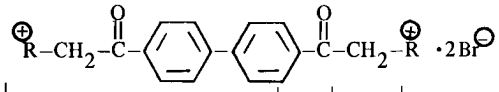
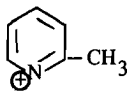
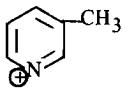
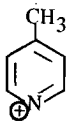
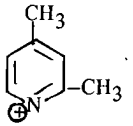
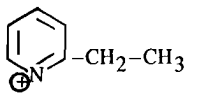
[a]Melting points are uncorrected.

[b]Compound is hemiketal according to IR spectra.

[c]Compound isolated as chloride salt.

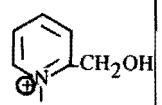
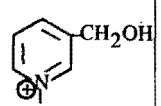
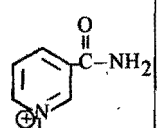
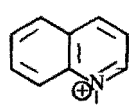
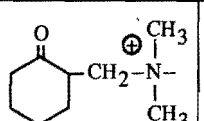
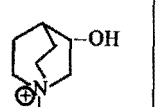
[d]Compound is tertiary amine, n_D^{23} 1.5470.

Table XI. Physical and Analytical Data of Symmetrical Bis-Quaternary Ammonium Compounds

Compound No.	EA No.	R	Reaction solvent	Empirical formula	Mol wt	Yield	mp[a]	Anal									
								Calcd					Found				
								C	H	Br	N	O	C	H	Br	N	O
					gm	%	°C										
																	
I_2[b]	4046		Pyridine	$C_{26}H_{22}I_2N_2O_2$	650	26	282-283	48.1	3.4	39.2	4.3		47.7	3.4	39.5	4.1	
I_3	4079		Dioxane	$C_{28}H_{26}Br_2N_2O_2 \cdot H_2O$	600	10	>300	56.0	4.7		4.7		55.9	4.7		4.4	
I_4	4080		Dioxane	$C_{28}H_{26}Br_2N_2O_2 \cdot 2H_2O$	618	9	265-266	54.4	4.9			10.4	54.6	4.9			10.8
I_5	4081		Dioxane	$C_{28}H_{26}Br_2N_2O_2 \cdot H_2O$	600	55	229-231	56.0	4.7		4.7	8.0	56.1	4.6		4.5	8.7
I_6	4919		2,4-Lutidine	$C_{30}H_{30}Br_2N_2O_2$	610	40	>170	59.0	5.0		4.6		59.0	5.1		4.6	
I_7	5049		2-Ethylpyridine	$C_{30}H_{30}Br_2N_2O_2$	610	44	260-261	59.0	5.0	26.2	4.6	5.2	59.5	5.3	26.1	4.6	5.4

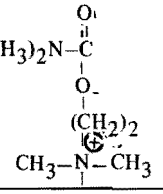
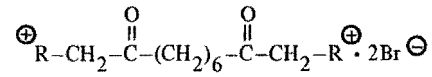
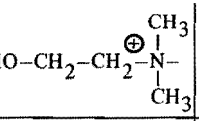
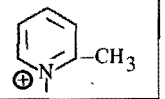
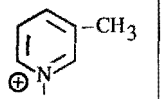
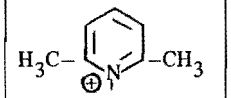
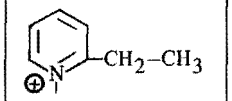
[a,b]See footnotes at end of table.

Table XI. Continued

Compound No.	EA No.	R	Reaction solvent	Empirical formula	Mol wt	Yield	mp[a]	Anal									
								Calcd					Found				
								C	H	Br	N	O	C	H	Br	N	O
					gm	%	°C										
I_8	4720		2-Pyridylcarbinol	$C_{28}H_{26}Br_2N_2O_4$	614	48	255-258	54.7	4.2	26.0			54.9	4.4	25.6		
I_9	4920		3-Pyridylcarbinol	$C_{28}H_{26}Br_2N_2O_4$	614	65	>160	54.7	4.3		4.6		54.7	4.7		4.8	
I_{10}	4050		Sulfolane	$C_{28}H_{24}Br_2N_4O_4 \cdot 1\frac{1}{2}H_2O$	671	60	307-309	50.4	4.1	24.0	8.4		50.5	4.1	23.8	8.6	
I_{11}	4881		Quinoline	$C_{34}H_{26}Br_2N_2O_2 \cdot H_2O$	672	55	218-220	60.7	4.2	23.7	4.2	7.1	60.6	4.5	23.3	4.4	7.0
I_{12}	3947		Tetrahydrofurane	$C_{34}H_{46}Br_2N_2O_4 \cdot \frac{1}{2}H_2O$	724	97	183-185	57.1	6.6			10.0	56.9	6.6			10.5
I_{13}	3827		Ethanol-water	$C_{30}H_{38}Br_2N_2O_4 \cdot H_2O$	668	96	235-237	53.9	6.0			12.0	53.9	6.1			11.5

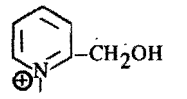
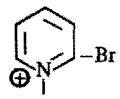
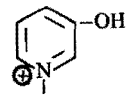
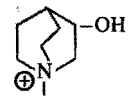
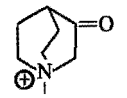
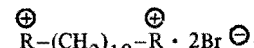
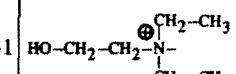
[a]See footnotes at end of table.

Table XI. Continued

Compound No.	EA No.	R	Reaction solvent	Empirical formula	Mol wt	Yield	mp[a]	Anal									
								Calcd					Found				
								C	H	Br	N	O	C	H	Br	N	O
					gm	%	°C										
I_{14}	3828		Ethanol-water	$C_{32}H_{48}Br_2N_4O_6 \cdot H_2O$	762	53	190-192	50.4	6.6	21.0			50.8	6.9	20.6		
																	
IV_1[b,c]	4075		Acetone	$C_{18}H_{38}I_2N_2O_4$	600	75	178-179	36.0	6.3		4.7	10.7	36.0	6.3		4.9	10.9
IV_2	4684		Acetronitrile	$C_{22}H_{30}Br_2N_2O_2$	514	70	229-231	51.4	5.8	31.1			51.4	5.7	30.9		
IV_3[b]	4076		Acetone	$C_{22}H_{30}I_2N_2O_2$	608	50	121-124	43.5	4.9		4.6	5.3	43.5	5.0		4.8	5.7
IV_4	4718		2,6-Lutidine	$C_{24}H_{34}Br_2N_2O_2 \cdot \frac{1}{2}H_2O$	551	42	240-245	52.3	6.4	29.0		7.3	52.4	6.2	28.9		7.5
IV_5	4913		2-Ethylpyridine	$C_{24}H_{34}Br_2N_2O_2$	542	36	188-189	53.1	6.5	29.5			52.6	6.7	29.1		

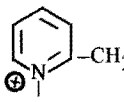
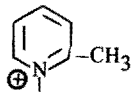
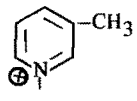
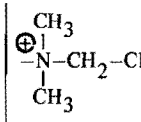
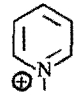
[a-c]See footnotes at end of table.

Table XI. Continued

Compound No.	EA No.	R	Reaction solvent	Empirical formula	Mol wt	Yield	mp[a]	Anal									
								Calcd					Found				
								C	H	Br	N	O	C	H	Br	N	O
					gm	%	°C										
IV_6	4831		2-Pyridylcarbinol	$C_{22}H_{30}Br_2N_2O_4$	546	80	154-156	48.3	5.5		5.1	11.7	48.3	5.4		5.2	12.1
IV_7	4691		2-Bromopyridine	$C_{20}H_{24}Br_4N_2O_2$	644	85	218-219	37.3	3.8	49.6			37.8	4.0	49.4		
IV_8	4833		Acetonitrile	$C_{20}H_{26}Br_2N_2O_4$	518	100	215-220	46.3	5.1	30.8		12.4	46.3	5.2	30.5		12.4
IV_9	4719		Acetonitrile	$C_{24}H_{42}Br_2N_2O_4$	582	83	181-183	49.4	7.2	27.5			49.4	7.2	27.1		
IV_{10}	4832		Acetone	$C_{24}H_{38}Br_2N_2O_4$	578	96	>226	49.8	6.6	27.7			49.7	7.0	27.4		
																	
V_1[c]	3831		Diethylamino-ethanol	$C_{22}H_{50}Br_2N_2O_2$	534	23	183-185	49.4	9.4	30.0			49.3	9.2	29.7		

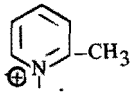
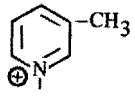
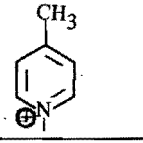
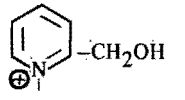
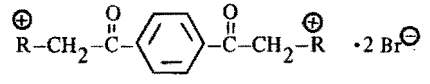
[a,c]See footnotes at end of table.

Table XI. Continued

Compound No.	EA No.	R	Reaction solvent	Empirical formula	Mol wt	Yield	mp[a]	Anal									
								Calcd					Found				
								C	H	Br	N	O	C	H	Br	N	O
					gm	%	°C										
V_4	3958		2-Pyridylcarbinol	$C_{22}H_{34}Br_2N_2O_2$	518	62	110-112	51.0	6.6	30.9			50.7	6.4	30.7		
		$\oplus R-(CH_2)_8-R\oplus \cdot 2\,Br\ominus$															
V_2	5199		2-Methylpyridine	$C_{20}H_{30}Br_2N_2$	458	15	262-263	52.4	6.6	34.9	6.1		52.2	6.7	35.0	6.0	
V_3	5147		3-Methylpyridine	$C_{20}H_{30}Br_2N_2$	458	22	133-134	52.4	6.6	34.9	6.1		52.1	6.9	34.7	5.9	
		$\oplus R-CH_2-C(=O)-C_6H_4-CH_2-CH_2-C(=O)-CH_2-R\oplus \cdot 2\,Br\ominus$															
VI_1[b]	5236		Acetonitrile	$C_{20}H_{34}Br_2N_2O_4$	526	100	218-220	45.6	6.5		5.3	12.2	45.8	6.8		5.4	12.3
VI_2	5298		Methanol	$C_{22}H_{22}Br_2N_2O_2$	506	27	295	52.2	4.4	31.6	5.5		52.1	4.5	31.5	5.4	

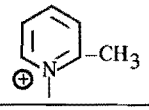
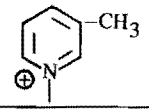
[a,b]See footnotes at end of table.

Table XI. Continued

Compound No.	EA No.	R	Reaction solvent	Empirical formula	Mol wt	Yield	mp[a]	Anal									
								Calcd					Found				
								C	H	Br	N	O	C	H	Br	N	O
					gm	%	°C										
VI_3	5195		Acetonitrile	$C_{24}H_{26}Br_2N_2O_2$	534	72	253	54.0	4.9	29.9	5.3		54.0	5.2	29.6	5.2	
VI_4	5244		Acetonitrile	$C_{24}H_{26}Br_2N_2O_2$	534	100	227-229	54.0	4.9	29.9	5.3		54.2	5.2	29.7	5.2	
VI_5	5245		Acetonitrile	$C_{24}H_{26}Br_2N_2O_2$	534	100	269-272	54.0	4.9	29.9	5.3		53.9	5.2	29.8	5.3	
VI_6	5243		Acetonitrile	$C_{24}H_{26}Br_2N_2O_4$	566	95	251-252	50.9	4.6	28.2	4.9	11.3	51.1	4.8	28.1	5.0	11.6
																	
VII_1[c]	5371		Acetonitrile	$C_{18}H_{30}Br_2N_2O_4$			200-201	41.9	6.2	31.1	5.4		42.3	6.1	31.3	5.4	

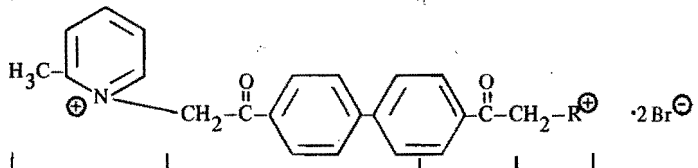
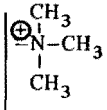
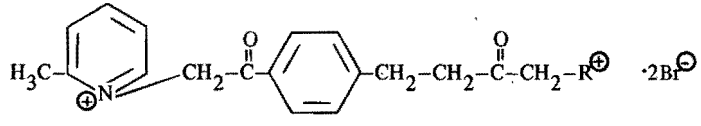
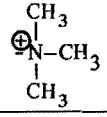
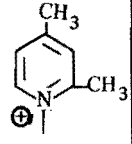
[a,c]See footnotes at end of table.

Table XI. Continued

Compound No.	EA No.	R	Reaction solvent	Empirical formula	Mol wt	Yield	mp[a]	Anal									
								Calcd					Found				
								C	H	Br	N	O	C	H	Br	N	O
					gm	%	°C										
VII_2	5382		Acetonitrile	$C_{22}H_{22}Br_2N_2O_2$ ½H_2O	515	36	198-202	51.1	4.5		5.4	7.8	50.6	4.7		5.2	7.7
VII_3	5299		Acetonitrile	$C_{22}H_{22}Br_2N_2O_2$	506	32	280	52.2	4.4	31.6	5.4		52.0	4.3	31.3	5.4	6.8

[a]Melting points are uncorrected.
[b]Compound isolated as iodide salt.
[c]Compound is hemiketal according to IR spectra.

Table XII. Physical and Analytical Data of Unsymmetrical Bis-Quaternary Ammonium Compounds

Compound No.	EA No.	R	Reaction solvent	Empirical formula	Mol wt	Yield	mp*	Anal					
								Calcd			Found		
								C	H	N	C	H	N
					gm	*%*	*°C*						
													
$VIII_1$	5490		Dimethyl formamide	$C_{25}H_{28}Br_2N_2O_2$	548	55	260	54.8	5.1	5.1	54.7	5.3	4.8
$VIII_2$	5448		Methanol	$C_{27}H_{24}Br_2N_2O_2 \cdot \frac{1}{2}H_2O$	577	44	295	56.2	4.4	4.8	56.5	4.7	4.7
													
IX_1	5352		Methanol	$C_{21}H_{28}Br_2N_2O_2$	500	25	175-180	50.4	5.6	5.6	50.4	5.7	5.6
IX_2	5345		Acetonitrile	$C_{23}H_{24}Br_2N_2O_2$	520	80	259-260	53.1	4.7	5.4	52.9	4.7	5.2
IX_3	5398		Methanol	$C_{25}H_{28}Br_2N_2O_2$	548	18	140-145	54.7	5.2	5.1	54.3	5.6	5.4

*All melting points are uncorrected.

for 3 days. The solid that precipitated was separated by filtration, dissolved in 10 ml of methanol, treated with activated carbon, and precipitated with ethyl acetate to give 1.4 grams (70% yield) of product, mp 229° to 231°C.

Anal for $C_{22}H_{30}Br_2N_2O_2$: Calculated: C, 51.4; H, 5.8; Br, 31.1. Found: C, 51.4; H, 5.7; Br, 30.9.

3. Unsymmetrical Quaternary Dihalides.

4-(2-Methylpyridinio)Acetyl-4'-(Trimethylammonio)Acetylbiphenyl Dibromide, EA 5490 (VIII).

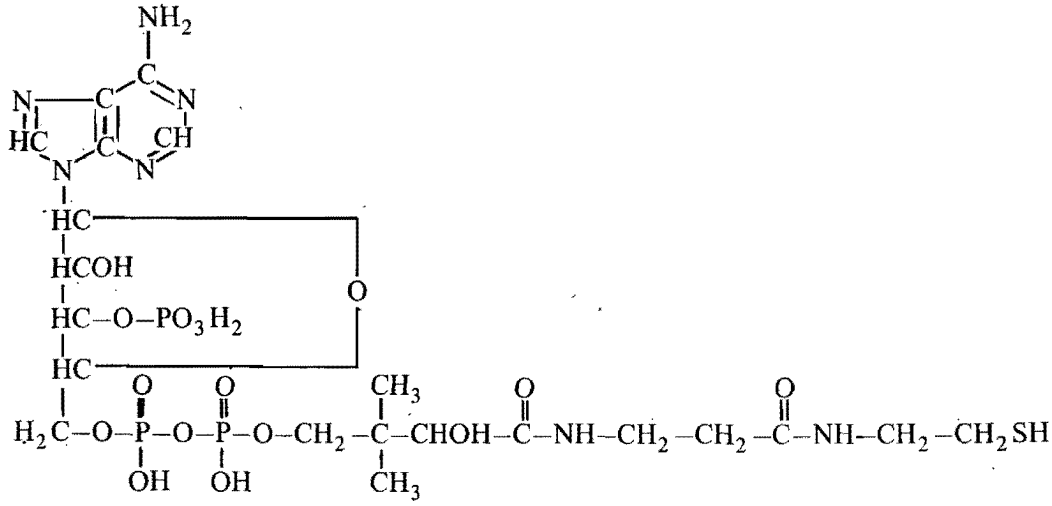
Anhydrous trimethylamine was passed through a solution of 0.98 grams (0.002 mole) of 4-(2-methylpyridinio)acetyl-4'-bromoacetylbiphenyl bromide in 20 ml of dimethylformamide at room temperature during 1 hour. The brown solid that precipitated was separated and recrystallized from methanol to give 1.2 grams (55% yield) of product, mp 230°C.

Anal for $C_{25}H_{28}Br_2N_2O_2$: Calculated: C, 54.8; H, 5.1; N, 5.1. Found: C, 54.7; H, 5.3; N, 4.8.

III. DISCUSSION AND RESULTS.

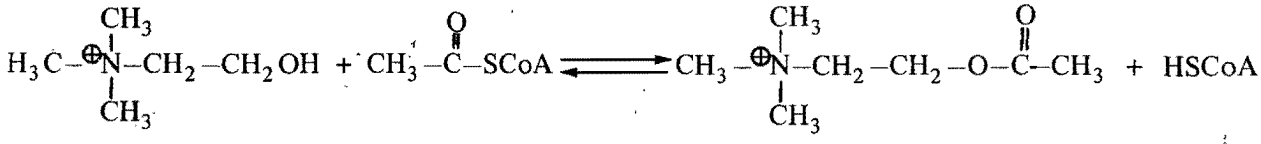
In accordance with the generally accepted neurohumoral theory of cholinergic transmission, acetylcholine (ACh) storage, release, and biosynthesis play essential parts in the complex sequence of events. To function as a chemical transmitter ACh must be present in nerve cells, ready to be released into the synaptic clefts when impulses reach the efferent nerve terminals. It must be replaced at a rate sufficient for transmission to occur repetitively in response to successive nerve impulses. ACh-rich particles have been isolated from brain tissues, and small vesicles within the axon of the neuron containing high concentrations of the transmitter substance have been identified by electron microscopy.[23-26] The release of ACh is believed to occur in multimolecular volleys.[27] The ability of the nerve cell to synthesize ACh depends foremost on the presence of the enzyme choline acetylase (ChAc). However, an additional factor, coenzyme A (CoA),[28-31] was found to take an essential part in the acetylation process of choline.

[23] Ralay, S. L., and Palade, G. E. J. Biophys. Biochem. Cytol. *1*, 69 (1955).
[24] DeRoberts, E. D. P., and Bennett, H. S. J. Biophys. Biochem. Cytol. *1*, 47 (1955).
[25] Del Castillo, J., and Katz, B. J. Physiol. (London) *128*, 157 (1955).
[26] Couteaux, R. Exp. Cell. Res. (Suppl.) *5*, 294 (1958).
[27] Fatt, P., and Katz, B. Exp. Cell.Res. *117*, 109 (1952).
[28] Nachmansohn, D., and Machado, A. L. J. Neurophysiol. *6*, 397 (1943).
[29] Nachmansohn, D., and Berman, M. J. Biol. Chem. *165*, 551 (1946).
[30] Lipmann, F., Novelli, G. D., Tuttle, L. C., and Buirard, B. M. J. Biol. Chem. *167*, 869 (1947).
[31] Novelli, G. D., Schmetz, F. J., Jr., and Kaplan, N. O. J. Biol. Chem. *206*, 533 (1954).



Coenzyme A

Acetylation presumably takes place in at least two enzymatic steps, that is, acetylation of the coenzyme A and subsequent transfer of the acetyl group to choline. The reaction catalyzed by ChAc can then schematically be presented as follows:



There is evidence[32] indicating the existence of "free" and "bound" ACh, the former being localized in the outer branchlets of the axon and more readily available for release into the synaptic cleft. The enzyme ChAc appears to be concentrated predominantly[10] in the same presynaptic vesicles that store ACh. For ACh biosynthesis it is then necessary to assume that choline must cross the outer membrane barrier of the axon and penetrate the vesicles of the nerve cell.

These findings indicate that, in principle, chemical agents can modify or completely block the normal presynaptic physiological process of impulse propagation by interference at a number of different loci in the complex biological reaction mechanism.

The intravenous doses at which the first pharmacological symptoms appeared (MED50's) and the lethal doses (LD50's) were determined. The data are compiled in tables I through IX. All compounds with the exception of the hemicholinium type derivatives produced typical parasympathomimetic responses similar to those of acetylcholinesterase inhibitors and depolarizing agents, such as miosis, lacrimation, fasciculations, and tremors. The times for onset and duration of action were generally relatively short.

In collaboration with T. Loomis,* AChE inhibitions of some of the methylpyridinium compounds were determined by the Michel ΔpH method using commercial semi-purified AChE as the enzyme source and acetylcholine chloride as the substrate. The values for the pI50's** are given as follows:

[32] Whitaker, V. P. Biochem. J. 72, 694 (1959).

*This study is in the process of being published in open literature.

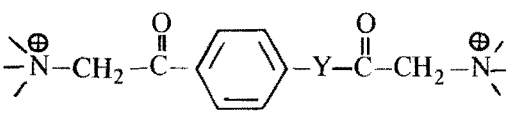
**Negative log molar concentration of inhibitor that produces 50% inhibition of the enzyme.

Compound No.	pI50
I_3	8.12
VI_3	7.27
VII_2	5.96
I_4	8.0
VI_4	6.98
VII_3	6.22

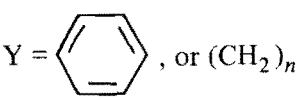
Benz and Long[19] determined the ID50's for AChE inhibition of compounds I_3 and I_4 and found ID50's of 6.1×10^{-9} *M* for I_3 and 1.9×10^{-8} *M* for I_4.

The hydroxydimethylammonium compounds that exist in the cyclized hemiketal forms II_1, III_1, IV_1, VI_1, and VII_1 affected the animals in essentially like manner as HC-3 (I_1). The predominant pharmacological signs were those indicating difficulties in respiration and weakness in muscular movements, such as decreased locomotor activity, decreased preening, mydriasis, decreased respiratory rate, and increased respiratory depth.

Relating the chemical structures to their biological activities, as measured by MED50's and LD50's, the general structure C evolves, depicting the common structural requirements that appear to be needed to attain potent hemicholinium type compounds, lethal pyridinium compounds, and other pyridinium compounds with large spreads between the effective and lethal doses. Furthermore, it becomes evident that certain moieties at the onium centers are instrumental in exerting very specific pharmacological effects. The *ortho*-methylpyridinio group provides good safety margins. The closely related *meta*-methylpyridinio group, on the other hand, is associated with high toxicities. The choline-like ethanoldimethylammonio group produces hemicholinium type activities.



C



The fact that the nature of the groups attached to the cationic heads plays a dominant role in determining the modes of action and the potencies of bis-quaternary ammonium compounds at the cholinergic synaptic junctions is very well known. Carbamates and phosphonates, for instance, give rise to AChE inhibitors, groups of small size produce depolarizing agents, and those of relatively larger size produce competitive blocking agents. However, the functional groups in the chain connecting the positively charged nitrogens generally contribute to a much lesser degree in the determination of modes of action. Rather, the bulk of the chain is the important factor influencing the affinities toward the receptor sites and, consequently, the potencies.

The specificity of the chain as schematically shown in general structure C is, therefore, very unusual and of great interest. On scrutinization of the data in tables I through IX, the required components leading to general structure C become apparent. One phenyl ring adjacent to one of the

two acetyl groups is essential for high potencies because the decamethylene-2,9-dione chain (table IV), possessing two acetyl functions but lacking aromatic rings, reduces potencies considerably. The polymethylene chains without the carbonyl groups (table V) reduce potencies even further. Both these chains drastically decrease the safety margins of the *ortho*-methylpyridinium compounds. When Y in general structure C represents a phenyl ring (table I) or ethylene (table VI), compounds of high potencies and of the aforementioned desired activities are obtained. The 1,4-phenylenediacetyl chain (table VII) in which Y is omitted gives compounds with activities similar to those in tables I and VI, but the potencies are somewhat lower. These results point toward the importance of a chain length of 14 to 16A.

On the basis of the unsymmetrical compounds containing only one *ortho*-methylpyridinio moiety (table VIII), which do not exhibit the high safety margins as their symmetrical analogs I_3 and VI_3, the specific moieties appear to be needed at each of the two onium centers. On the other hand, unsymmetrical compound IX_3, in which an *ortho*-methylpyridinio group is attached at one end of the chain and a *para-ortho*-dimethylpyridinio group at the other, equals its symmetrical analog VI_3 in regard to safety margin. The MED50 and LD50, however, are one order of magnitude lower.

The necessity for two positively charged nitrogens is supported by the finding that the structurally closely related mono-quaternary methylpyridinio compounds (tables II and III) produce neither high toxicities nor large ratios between the LD50's and MED50's. The mono-quaternary members in the series of compounds in table II represent half the molecules of their bis-quaternary analogs in table I. The mono-quaternary compounds in table III containing biphenyl moieties are comprised of even a greater part of the bis-quaternary molecules presented in table I. In the case of the mono-quaternary hemicholiniums in tables II and III, the distinctive pharmacological symptomology is maintained, but the potencies as measured by the MED50's and LD50's are greatly reduced.

The fact that the same rather specific structural characteristics that have been shown to be needed for potent hemicholinium type activities are also required for potent lethal actions and for unusually great safety margins strongly suggests that both types of the methylpyridinium compounds interfere presynaptically with the processes involved in the propagation of neural impulses. Moreover, in view of the numerous published structure-activity relationship studies, the diverse pharmacological results of compounds that differ only in the position of a methyl group in the pyridine ring do not appear to be explicable on the basis of only postsynaptic modes of action.

At first sight a mechanism whereby the methylpyridinium compounds simultaneously react with acetylcholinesterase and the acetylcholine receptors at the excitable part of the sarcoplasmic membrane seems plausible. The *ortho*-methylpyridinio moiety, being somewhat sterically hindered,[33,34] could prevent close approach to the ACh receptors for the molecule to act as a depolarizing agent, but close enough to act as curare-like competitive antagonist, thereby counteracting the effects of AChE inhibition. Such dual action mechanism could account for larger ratios between the LD50's and MED50's. The *meta*-methylpyridinio moiety, not being sterically hindered, could act as an agonist. Simultaneous depolarizing and AChE inhibiting actions could explain high toxicities. If steric hindrance, however, is presumed to be the dominant factor governing the interaction with the ACh receptors, it is difficult to reconcile with the fact that the *ortho*-ethylpyridinium compound I_7, being sterically more hindered than the methyl analog I_3, shows a safety margin 57 times smaller. Furthermore, in the above rationale, the effects of steric hindrance are disregarded in the interaction with the active sites of AChE, which are believed to be of nearly identical composition as the ACh receptors at the sarcoplasmic membrane.[35] The high

[33] Sommer, H. Z., and Jackson, L. L. J. Org. Chem. *35*, 1558 (1970).

[34] Sommer, H. Z., Lipp, H. I., and Jackson, L. L. J. Org. Chem. *36*, 824 (1971).

[35] Ehrenpreis, S. *In* Drugs Affecting the Peripheral Nervous System. Alfred Burger, ed. Vol 1, ch I. Marcel Dekker, Inc., New York, New York. 1967.

lethal potencies of the *meta*-methylpyridinium compounds can be disputed on the grounds that the analogs IV_4 and V_3, which structurally more closely resemble depolarizing agents, are considerably less toxic than I_4, VI_4, and VII_4.

In a very broad sense, the pharmacological effects exerted by compounds interfering with neuromuscular impulse transmission can be looked upon as being caused in the final result by changes in ACh concentrations in the synaptic clefts. Larger amounts of ACh available for interaction with the receptors at the postsynaptic membranes result in overstimulation, such as maintained depolarization, of the muscles and eventual collapse of muscular functions; smaller concentrations give rise to muscular weakness and eventual paralysis. It is proposed that the high potencies of the *ortho*-methylpyridinium compounds, as measured by the MED50's, originate from postsynaptic AChE inhibition and that the large safety margins are brought about by simultaneous presynaptic actions blocking ACh release. The increase of ACh concentrations in the synapses created by enzyme inhibition is counteracted by a decrease of ACh influx from the nerve endings. The high toxicities of the *meta*-methylpyridinium compounds are proposed to be caused by simultaneous postsynaptic AChE inhibition and presynaptic stimulation of ACh release, resulting in cumulative effects toward increase of ACh concentrations in the myoneural synapses.

Based on the AChE inhibition and other pharmacological data presented in this study, it seems reasonable to conclude that the presynaptic active sites are very similar in composition to the postsynaptic ACh receptors and the active sites of AChE in respect to the anionic centers and spacings of the electrical charges. The presynaptic sites, however, appear to differ considerably in their makeup in the areas surrounding the charges. In a logical extension, bis-quaternary compounds, complimentary in structure, on reaching the cholinergic synapses could potentially interact with the anionic centers of a number of active sites. The interactions can occur simultaneously or sequentially, and the in vivo observable pharmacological symptoms should then be considered as the sum net result of the different interactions affecting the normal physiological process at the various loci. The local effects depend on the individual rates of interaction; they, in turn, are influenced by the affinities of the chemical agents toward the individual physiological sites. Moreover, the accessibilities of the particular sites to the drugs and the different concentrations required to trigger the particular local pharmacological responses play important roles and add to the complexity of the modes of action of chemical compounds in biological systems. All these factors contribute in the determination of the times of onset of action, duration, and potencies.

Taking into consideration the evidence derived from the published biological studies with hemicholiniums and, hence, presuming presynaptic activities, the methylpyridinium compounds can interfere with the normal physiological processes in a number of ways. These compounds can interfere with the crossing of choline through the membranes of the axons; the penetration of ACh into the storage vesicles; the probable, but not yet established, active centers at the nerve terminals that are involved in the trigger mechanism of ACh release; the ACh concentration mechanism; and the ACh biosynthesis by inhibition of the enzyme ChAc or coenzyme A. In the latter, the addition reaction of the sulfhydro groups to the carbonyls of the chain, as well as hydrogen bonding, can be envisioned. Taking the time factors of onset of action, duration, and times to death into account, the probable number of presynaptic actions contributing to the final specific pharmacological activities narrows down markedly. The onset times of parasympathomimetic symptoms at the MED50 dose levels, as well as at the LD50 levels of the *ortho*-methylpyridinium compounds I_3, VII_3, and VII_2, have been found to be 2 to 3 minutes. The times to death at the LD50 doses averaged 4 minutes. Based on these findings, it follows that the counteraction of presynaptic decrease of ACh release producing the relatively great safety margins has to take place during similar time periods. It is proposed, therefore, that the *ortho*-methylpyridinium compounds interfere directly with the release mechanism of ACh, thereby counteracting the higher concentrations of ACh that result from AChE inhibition. Because of lack of convincing biological evidence, it can be speculated only that a blocking of the trigger mechanism for release of ACh at the nerve membranes or at the vesicles storing "free" ACh might occur.

The onset time of the cholinergic effects of the *meta*-methylpyridinium compounds I_4, VI_4, and VII_3 are similar to those of the *ortho* analogs. The times to death at the LD50 dose levels, however, are longer, i.e., 10 to 15 minutes. The animals maintain the cholinergic symptoms until the times of death, and the surviving animals show parasympathomimetic effects during 1 to 2 hours. Thus, it can be deduced that the postulated presynaptic increase of ACh release does not result from direct stimulation on the ACh release mechanism at the nerve endings; rather, the increase of ACh release originates by stimulation at more distant sites, possibly at sites in the central nervous system. It is proposed that the high toxicities of the *meta*-methylpyridinium compounds are caused by postsynaptic AChE inhibition and, presynaptically, by increased ACh release originating predominantly from actions at sites remote from the nerve terminals.

IV. CONCLUSIONS.

Nine selected series of quaternary ammonium compounds were synthesized to study relationships between their chemical structures and their biological activities. A general structure is presented that indicates certain common chemical features required to obtain potent hemicholiniums, lethal pyridinium compounds, and other pyridinium compounds with relatively great differences between the effective and lethal doses. A new hemicholinium compound (EA 5236), more potent than the original hemicholinium (HC-3), was obtained. General chemical characteristics regarding the compositions of the presynaptic active sites participating in cholinergic neuromuscular impulse transmission are postulated. Possible mechanisms of action of *ortho*- and *meta*-pyridinium compounds meeting specific structural requirements are proposed.

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13. ABSTRACT

Nine selected series of quaternary ammonium compounds were synthesized to study relationships between their chemical structures and their biological activities. A general structure is presented indicating certain common chemical features required to obtain potent hemicholiniums, lethal pyridinium compounds, and other pyridinium compounds with relatively great differences between the effective and lethal doses. A new hemicholinium compound more potent than the original hemicholinium (HC-3) was obtained. General chemical characteristics regarding the compositions of the presynaptic active sites participating in cholinergic neuromuscular impulse transmission are postulated. Possible mechanisms of action of *ortho*- and *meta*-pyridinium compounds meeting specific structural requirements are proposed.

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Quaternization
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Hemicholiniums
Lethal compounds
Ortho-methylpyridinium compounds
Meta-methylpyridinium compounds
Minimal effective doses (MED50's)
Lethal doses (LD50's)
Safety margins
Structure-activity relationships
Cholinergic mechanisms
Acetylcholine
Acetylcholine biosynthesis
Acetylcholine receptors
Acetylcholinesterase
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